

Intercropping effect on root growth and nitrogen uptake at different nitrogen levels

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Abstract

Aims

Intercropping legumes and non-legumes may affect the root growth of both components in the mixture, and the non-legume is known to be strongly favored by increasing nitrogen (N) supply. The knowledge of how root systems affect the growth of the individual species is useful for understanding the interactions in intercrops as well as for planning cover cropping strategies. The aim of this work was (i) to determine if different levels of N in the topsoil influence root depth (RD) and intensity of barley and vetch as sole crops or as an intercropped mixture and (ii) to test if the choice of a mixture or the N availability in the topsoil will influence the N uptake by deep roots.

Methods

In this study, we combined rhizotron studies with root extraction and species identification by microscopy with studies of growth, N uptake and ^{15}N uptake from deeper soil layers, for studying the root interactions of root growth and N foraging for barley (*Hordeum vulgare* L.) and vetch (*Vicia sativa* L.), frequently grown in mixtures as cover crops. N was added at 0 (N0), 50 (N1) and 150 (N2) kg N ha⁻¹. The roots discrimination relying on the anatomical and morphological differences observed between dicots and monocots proved to be a reliable method providing valuable data for the analysis.

Important Findings

The intercrop and the barley attained slightly higher root intensity (RI) and RD than the vetch, with values around 150 crosses m⁻¹ and 1.4 m, respectively, compared to 50 crosses m⁻¹ and 0.9 m for the vetch. At deep soil layers, intercropping showed slightly larger RI values compared to the sole-cropped barley. The barley and the intercropping had larger root length density (RLD) values (200–600 m m⁻³) than the vetch (25–130) at 0.8–1.2 m depth. The topsoil N supply did not show a clear effect on the RI, RD or RLD; however, increasing topsoil N favored the proliferation of vetch roots in the intercropping at deep soil layers, with the barley:vetch root ratio ranging from 25 at N0 to 5 at N2. The N uptake of the barley was enhanced in the intercropping at the expense of the vetch (from ~100 mg plant⁻¹ to 200). The intercropped barley roots took up more labeled nitrogen (0.6 mg ^{15}N plant⁻¹) than the sole-cropped barley roots (0.3 mg ^{15}N plant⁻¹) from deep layers.

Keywords: grasses, legumes, cover crops, crop mixture, root discrimination, labeled N

INTRODUCTION

Intercropping, the simultaneous cultivation of two or more species on the same physical space, presents some possible benefits including increased yield, yield stability and improved forage quality and soil fertility (Lithourgidis *et al.* 2011). The achievement of these benefits relies on the complementary and non-competitive utilization of the growth resources by

the intercropped elements. Root foraging plays an important role in nutrient uptake (Hutchings and de Kroon 1994) but has not been widely studied yet due to the complexity of belowground interactions and the technical difficulties to carry out these studies. The root foraging strategy may vary in different plant species and in relation to the distribution and mobility of the nutrients, increasing the root density in nutrient-rich areas (Linkohr *et al.* 2002). Likewise, roots

can exhibit different responses in the presence of a neighbor due to their plasticity (Hodge 2004); e.g. they can avoid other roots if the resources are not abundant (Schenk *et al.* 1999) or overproduce roots in an attempt to increase competitiveness (Izaurrealde *et al.* 1992). Bartelheimer *et al.* (2008) found a correlation between neighbor plants' root sizes and their competitive effect on their target plants' nitrate uptake. Thorup-Kristensen (2001) and Kristensen and Thorup-Kristensen (2004) showed the importance of the root depth (RD) and distribution for the ability of a root system to absorb soil N. Thus, it would be valuable to further explore the influence of intercropping on the root foraging, deep rooting and on nutrient uptake.

Intercropping benefits can in many cases be attributed to reduced competition by at least one component of the mixture (Vandermeer 1989). In legume/non-legume mixtures, the non-legume will benefit from the reduced competition for N with the legume. One important reason for the reduced competition is that the legume can use biological fixation as an alternative source of N. Measuring the root growth of red beet accompanied either by an intraspecific or an interspecific (clover or faba bean) neighbor, Tosti and Thorup-Kristensen (2010) concluded that the mixture enhanced the root growth of the non-legume and also its biomass and the final N uptake. The increase in N availability for the non-legume can also be a result of facilitative interactions by roots such as the processes of rhizodeposition and N-transfer (Jensen 1996a). Increased N fertilization is usually observed to favor mainly the non-legume, which is a stronger competitor and thus gets a bigger share of the available N (Corre-Hellou *et al.* 2006; Jensen 1996b). In this case, the legume might also be affected because the higher N availability in the soil may inhibit N fixation (Chu *et al.* 2004). Other authors attribute a part of the larger N uptake of the non-legume to higher root length densities (RLDs) in the mixtures compared with the legume, improving the competitive abilities of the non-legume for soil N (Corre-Hellou *et al.* 2007; Li *et al.* 2006).

Mixtures of cereal and legumes can increase the N use efficiency in the agrosystem through their complementary usage of the N (Vandermeer 1989). While the legume can supply N from an external source provided by the biological fixation, the cereal can accumulate N and reduce the risk of leaching losses (Ranells and Waggar 1997). Tosti *et al.* (2012) conducted an experiment with barley and vetch showing that while the cereal attained elevated growth rates during the cold season depleting the N in the soil, the vetch increased its growth in spring using N fixation. Crop mixtures have been used in cover cropping, and legume/cereal mixtures have been particularly successful (Kuo and Sainju 1998; Teasdale and Abdul-Baki 1998). This could represent an interesting complementary performance when growing cover crops because despite the increased costs for buying cover crops seeds, the extra N accumulation due to intercropping with a legume may compensate for the extra cost. When the cover crops are aimed at protecting the accumulated N in the soil from

leaching and transfer it to the succeeding cash crop, the intercropping with legumes can perform a substantial modification in the composition of the plants, decreasing its C/N ratio and increasing the N mineralization and N availability for the following crop. Deep roots can also improve N use efficiency in crop rotations. The knowledge on root growth and N uptake from deeper soil layers can be used in the design of crop rotations growing deep-rooted cover crops extending their roots to below the root zone of the main crops (Kristensen and Thorup-Kristensen 2004). In this sense, cereal and legume species intercropped may produce deeper and more intense roots than sole cropped, increasing the ability of the mixture to extract N from the soil. The initial amount of N in the soil may affect the root growth and also the N uptake. There is a need for further research on the behavior of roots in cereal-legume mixtures and the importance of their interactions in the N use efficiency of the plants.

The aim of this work was (i) to determine if different levels of N in the topsoil influence RD and intensity of barley and vetch as sole crops or as an intercropped mixture and (ii) to test if the choice of a mixture or the N availability in the topsoil will influence the N uptake by deep roots.

MATERIALS AND METHODS

Experimental site, plant material and treatments

The experiment was conducted under greenhouse conditions at the Copenhagen University in Taastrup (Denmark) between the months of August and October in 2012. Seedlings of barley (*Hordeum vulgare* L. cv Hispanic) and vetch (*Vicia sativa* L. cv Aitana) were transplanted on 21 August 2012 to rhizotron cylinders similar to the ones used by Tosti and Thorup-Kristensen (2010). The rhizotrons had an inner diameter of 0.104 m and 2 m height. Two plants were placed with 35 mm distance to each other at the top of each rhizotron, as sole crop or in intercrop. The plants were grown at three different levels of nitrogen supply. The initial nitrogen contained in the soil was increased by adding, respectively, 0, 50 and 150 kg N ha⁻¹. There were 27 experimental units (nine treatments and three replicates) arranged in a randomized block design. Temperature was monitored during the whole cycle with readings every 10 min and accumulated daily average temperature from transplanting (day °C) was calculated with a base temperature of 0°C.

Growing medium

The growing medium consisted in a repacked sandy soil, mixed from two types of soil taken from the research fields in Taastrup. To ensure a low N level, the tubes were filled with a soil taken from the lower horizon (0.2–0.6 m) of an organic arable field. The texture was 3.9% clay, 4.7% silt, 33.3% fine sand and 57.1% coarse sand (0.2–2 mm Ø). The soil had 7.7 mg kg⁻¹ of NO₃-N, 9 mg kg⁻¹ of P and 1% of humus. The upper 250 mm of the rhizotrons were filled afterward with material taken from the upper horizon of the same soil, containing

10.4 mg kg⁻¹ of NO₃-N, 12 mg kg⁻¹ of P and 2.5% humus. No fertilizers were added before planting. Both soils were sieved, homogenized and moistened until field capacity. The cylinders were filled by hand while standing on a vibratory platform powered by a NTK 25 AL piston oscillator. The oscillator was set at 6 bar and an amplitude of 5.8 mm. The tubes were filled and vibrated during an average time of 8 min. The compacting produced led to an approximate bulk density of 1.5 Mg m⁻³ inside the tubes. An opaque plastic cover prevented the incidence of light in the tubes, being removed only when the measurements were performed.

Water supply and N levels

The rhizotrons were maintained at field capacity during the experiment by manual watering applied at the top. The plants were given 200 ml four times, which is equivalent to ca. 96 mm rainfall. The first irrigation was followed by 25 ml of nutrient solution containing NH₄NO₃ in the amounts of 0, 50 and 150 kg N ha⁻¹ (based on the surface area of the tubes) for the low, medium and high nitrogen treatment, respectively. The medium treatment represented an addition of 0.04 g N per tube while the high meant an increase of 0.13 g N in each rhizotron.

Root growth measurements

A grid (20×20 mm) squares was printed on transparent plastic sheets and placed on the surface of the rhizotrons for the root measurements. The root intensity (RI) was assessed by counting the number of crossings per meter grid line (Thorup-Kristensen 2001). RD was estimated as the deepest root crossing detected. A total of six root countings were made during the experiment.

¹⁵N injection

After the final root measurements, leaf samples were collected for measuring the natural ¹⁵N enrichment in the plants (Peoples *et al.* 1995) based on the δ¹⁵N (‰) determination (Europa Scientific 20-20 IRMS Analyser®, Crewe, UK), in order to ensure that correction could be made in the calculations for the initial differences between the treatments. Subsequently, a ¹⁵N-enriched solution was injected into the tubes at a depth of 0.9 m, chosen to be at the deepest part, but still within the root zone of vetch and thereby well within the root zone of barley. This made sure that roots within all the treatments were present in the labeled soil layer and able to absorb ¹⁵N. With the purpose of getting a uniform distribution of the ¹⁵N injected, four holes of 6 mm diameter were made on the surface of each rhizotron by means of a drilling machine. The holes were made equidistant to each other, 0.9 m below the soil surface. To prevent the loss of soil and water from the tubes, the cavities were covered before and after the injection with an adhesive tape. Sixteen milliliters of the ¹⁵N solution, containing 7.9 mmol l⁻¹ NH₄¹⁵NO₃ (98% atom ¹⁵N), was injected into each soil column with a syringe providing 3.7 mg ¹⁵N to each rhizotron. The amount was chosen to be large enough to prevent the plants from assimilating all of it during the following week and thereby to reduce direct

competition for it. One week after the injection the plants were harvested and their biomass accumulation and ¹⁵N content measured.

Aboveground plant sampling and analysis

Aboveground biomass was harvested 77 days after sowing (GS-34, Lancashire *et al.* 1991) and dried in an oven at 70°C for 48 h. The plant material obtained was weighed and subsamples were analyzed to determine its N content by the Dumas method (LECO CHNS-932® Analyser, St. Joseph, MI, USA) and the δ¹⁵N (‰). The total N uptake per plant was calculated as the product between the N concentration and the biomass, which in the case of the monocropped rhizotrons was calculated as half total amount in the tube. The ¹⁵N uptake was corrected for the natural abundance of atmospheric ¹⁵N, which was taken as 0.3663, and calculated according to Equation (1):

$$^{15}\text{N}_{\text{upt}} = \left(\frac{\text{Atom}\%^{15}\text{N} - 0.3663}{100} \times \text{N}_{\text{upt}} \right) \quad (1)$$

where ¹⁵N_{upt} is the ¹⁵N uptake value calculated for each plant, Atom% ¹⁵N is the ¹⁵N concentration in a plant sample and N_{upt} is the total N uptake per plant.

Root sampling and analysis

After sampling of the aboveground material, the soil layer between 0.8 and 1.2 m depth was extracted from each tube to wash the roots out of the soil and sieved through a 2 mm mesh. The roots were preserved in a solution of ethanol (15%).

Roots samples from intercropping treatments were cut into 10 mm segments and mixed thoroughly. Fifty root pieces from each sample were arranged in 2% agarose divided in five rubber molds. Solidified agar blocks were trimmed and fixed for 4 h in Karnovsky's fixative (5% glutaraldehyde, 4% paraformaldehyde, 0.1 M sodium cacodylate buffer) and washed in cacodylate buffer at pH 7.3. Following washing in water, the samples were dehydrated in a graded acetone series, infiltrated in increasing ratio of acetone to Spurr resin and embedded in Spurr resin in flat molds. The resin was polymerized in an oven at 60°C for 8 h. Root cross-sections of 3 μm were cut with a histodiamond knife using a Reichert-Jung/LKB Supernova ultramicrotome and collected on microscope slides. Slides were stained with the fluorescent dye coriphosphine O (excitation peak 546 nm, emission peak 630 nm) and observed in a Nikon Eclipse 80i light and fluorescence microscope at ×20 objective. The number and arrangement of xylem elements within the root stele were used as characters for discrimination between the two species. Roots from vetch had either three or four groups of xylem elements typical for dicot species. Barley had a grass anatomy with either one or two central xylem element surrounded by numerous (>5) peripheral xylem elements. Contrast adjustments were carried out to improve clarity of images but did not alter overall appearance. Final image processing, cropping and mounting of the images were done with Adobe Photoshop CS2 and Illustrator CS2.

The RLD was analyzed from images obtained with an EPSON® Scanner by means of Winrhizo® software. The RLD results of the intercropping treatment were multiplied by the roots ratios obtained at different N levels in order to estimate a RLD value for each of the species intercropped. An estimate of deep root activity was calculated by dividing the ^{15}N uptake of the plants by their root length (m).

Statistical analysis

The general linear model procedure of SPSS was used to test the effects of the cropping method and the N levels on the data. Two-way and one-way analyses of variance (ANOVAs) were performed, comparing the results based on least significant difference values derived from the ANOVA. Tests with $P < 0.05$ were considered statistically significant.

RESULTS

Root measurements

Root intensity

The RI measured in four depth layers (each 0.4 m), as well as in the whole soil column, is shown in Fig. 1. The barley and the intercrop grew more roots than the vetch already from the early measurements. The sole-cropped cereal attained moderately higher values than the intercrop in the shallower layers and in the global measurements at the lower N levels (N0 and N1), while at the highest N level (N2) they were reduced, or even inverted with higher RI in the intercrop. In the deepest soil layers, the intercropping produced slightly more roots than the monocrops, except in the case of the moderate level

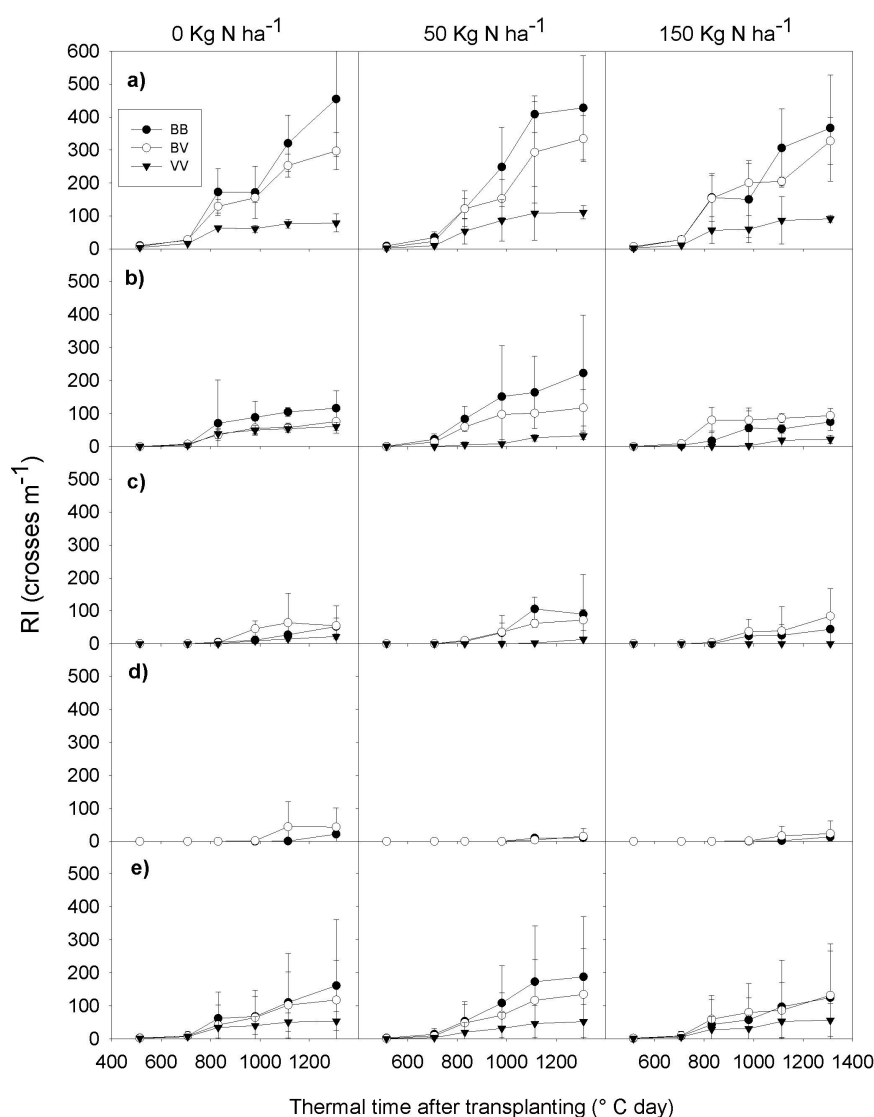


Figure 1: RI at (a) 0–40, (b) 40–80, (c) 80–120, (d) 120–160 cm depth and (e) the whole column at low (0 kg N ha^{-1}), medium (50 kg N ha^{-1}) and high (150 kg N ha^{-1}) N supply. BB, barley monocrop; BV, barley–vetch intercrop; VV, vetch monocrop. Bars represent standard errors.

of N (N1), where the barley still reached similar values as the intercropping.

Root depth

Barley and intercropped barley/vetch had higher RD penetration rates than the vetch at all N levels (Fig. 2). There were no significant differences in RD among the crops at low N supply until the end of the experiment, but the barley and the intercropping showed a tendency of deeper rooting than vetch. At higher N soil levels, the barley and the intercrop tended to attain deeper rooting already at early stages. Despite the lack of statistical differences among N levels, at all of them the barley and the intercrop had similar and much faster depth development than vetch during the whole experimental period.

Discrimination between species

Despite the fact that many barley roots had a diameter of $\sim 300\ \mu\text{m}$, while vetch roots could be up to $800\ \mu\text{m}$ diameter, a large portion of roots could not be discriminated by simple characters like root diameter and thickness of cortex as seen from the stereomicroscope images (Fig. 3a–d). However, following fixation, embedding and sectioning, the morphological and anatomical characteristics of the root cross-sections could be analyzed with fluorescence microscopy (Fig. 3e–h). Barley roots had a typical monocot (grass) anatomy. Main part of the barley roots were from the seminal (primary) root system recognized by their single central metaxylem vessel encircled by five to eight peripheral metaxylem elements and numerous smaller protoxylem elements. A smaller amount of roots were assessed as adventitious roots characterized by two central metaxylem elements, seven to nine peripheral metaxylem elements and many protoxylem elements. Vetch roots were identified by the stele morphology typical for dicots here with either three or four xylem poles and a more or less lignified central region. In general, roots were from different stages of development, the mature roots from both species had well-developed secondary wall thickening in the xylem tissue as well as a fully developed endodermis with deposition of suberin and lignin in the cell walls. The results showed that features such as number and arrangement of xylem vessels

were helpful for discrimination of the two species (Fig. 3g and h). In all the samples analyzed, the barley roots were outnumbering the vetch roots, but the fraction of vetch roots increased with increasing level of N (Fig. 4).

Root length density

At the soil layer between 0.8 and 1.2 m, the intercropped barley attained higher RLD values ($399\text{--}612\ \text{m m}^{-3}\ \text{plant}^{-1}$), reaching almost the double the RLD of the monocropped barley at all N levels ($220\text{--}321\ \text{m m}^{-3}\ \text{plant}^{-1}$), and higher values than the vetch at any case ($25\text{--}136$) (Table 1). At increasing N supply, the RLD of barley was reduced, whereas RLD of vetch tended to be slightly increased, though the increase was not statistically significant.

Biomass accumulation, N concentration and N uptake

The vetch reduced its biomass in intercropping producing only $\sim 1\ \text{mg plant}^{-1}$ (Table 2), while the barley increased its growth and reached $4.2\text{--}4.6\ \text{mg plant}^{-1}$. The N level did not have any effect on the aboveground biomass accumulation, and only a non-significant tendency for increased N concentration (Table 3) and uptake (Fig. 5) with increased N fertilization was seen.

The N concentration in the intercropped barley was always slightly higher than in the monocrop, with differences ranging from $2\ \text{mg kg}^{-1}$ at N1 level to $6\ \text{mg kg}^{-1}$ at N2. On the other hand, the vetch always had higher values when grown in monocrop compared to intercropping (differences were between 2 and $3\ \text{mg kg}^{-1}$).

The intercropped barley always took up most N ($158\text{--}193\ \text{mg plant}^{-1}$), reaching twice the amount of the monocropped barley at N2. The plant N uptake response to topsoil N availability was higher for the intercrop barley than for the other treatments. The intercropped vetch always showed the lowest N uptake. Both monocropping treatments showed values between these two.

The total ^{15}N uptake only reached a maximum of 35% of applied ^{15}N , indicating that the uptake of one plant did not have a strong effect on ^{15}N availability for the other plant in the tube. The intercropped barley showed the highest ^{15}N

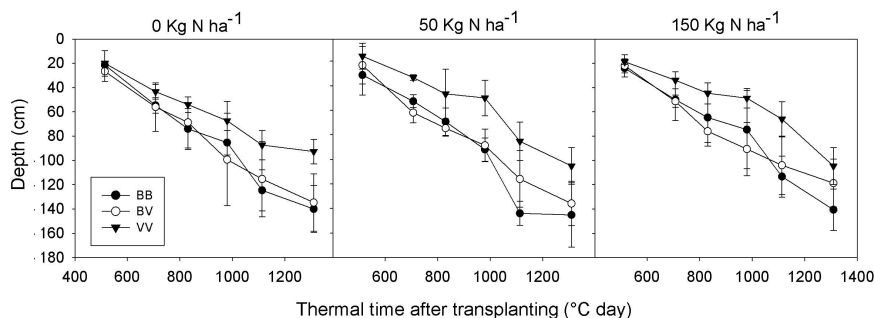


Figure 2: RD reached by the low ($0\ \text{kg N ha}^{-1}$), medium ($50\ \text{kg N ha}^{-1}$) and high ($150\ \text{kg N ha}^{-1}$) N supply in six sampling dates. BB, barley monocrop; BV, barley–vetch intercrop; VV, vetch monocrop. Bars represent the mean standard errors.

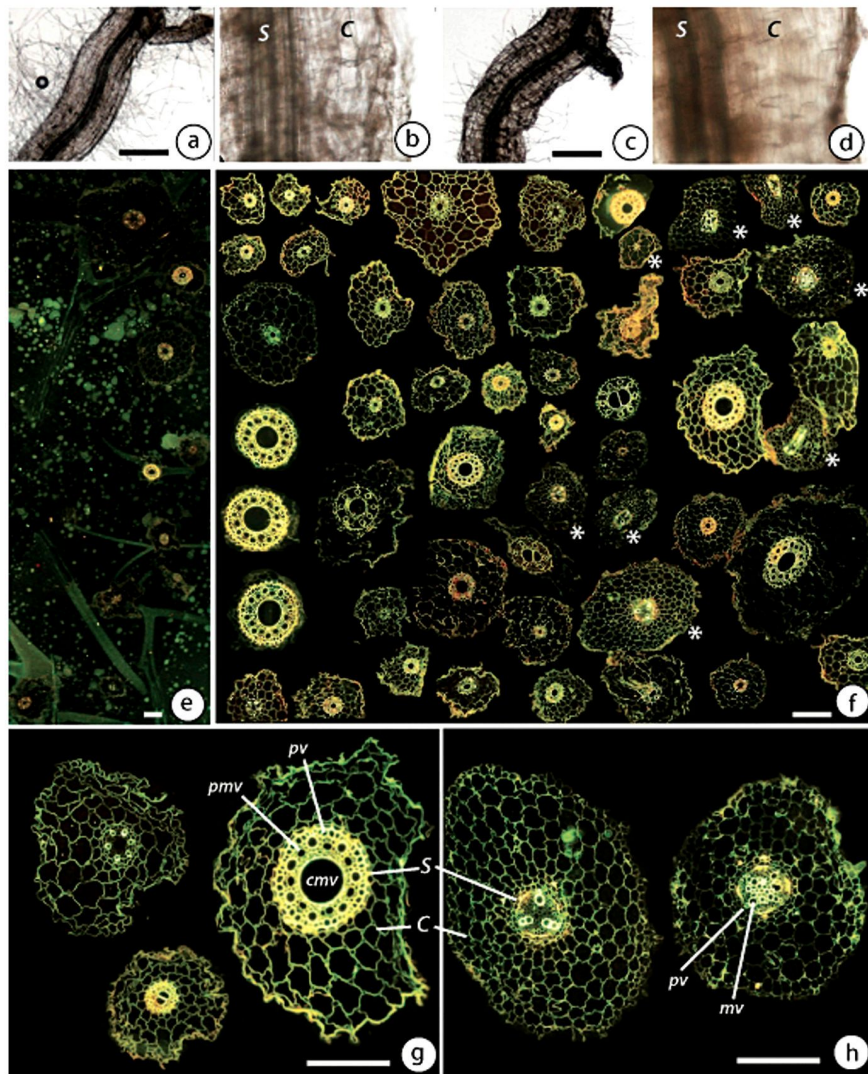


Figure 3: discrimination between barley and vetch root samples using morphological features and microscopy. (a–d) Stereomicroscopy on intact root tissue. (a) and (b) Barley root. (c) and (d) Vetch root. (e–h) Fluorescence microscopy on root cross-sections following fixation, resin-embedding and staining with coriphosphine. (e) Example of entire section containing 10 roots. (f) Collage of root sections from a sample with high N supply, image enhanced and pasted into one image. Sections marked by white asterisks represent vetch, all other sections are barley. Frequently occurring root types in samples from barley (g) and vetch (h). c, cortex; cmv, central metaxylem vessel; mv, metaxylem vessels; pmv, peripheral metaxylem vessel; pv, protoxylem vessel; s, stele.

uptake at the end of the experiment, taking up between 0.56 and 0.67 mg plant⁻¹ (Fig. 6). The barley in monocrop showed similar values to the vetch at N0, but higher values than the legume with increased N supply. The vetch in intercrop always had the lowest uptake (0.0004–0.0078 mg plant⁻¹). The intercropping reduced the ¹⁵N uptake per meter vetch root from 0.2–0.4 to 0.01–0.05 mg ¹⁵N m⁻¹ root, while the barley slightly increased its uptake with 0.3–0.5 compared to 0.2–0.4 mg ¹⁵N m⁻¹ root in monocrop (Table 4).

DISCUSSION

We know that in legume/non-legume mixtures, the non-legumes are normally dominating and this domination becomes

stronger with higher N levels (Ofori and Stern 1987a). Previous studies have indicated that much of this dominance is due to the non-legumes dominating in the root competition (Corre-Hellou *et al.* 2007; Li *et al.* 2006). In this study, we focused on a specific aspect of this and found that in a barley/vetch mixture, the barley was dominant in root growth, as shown through microscopic discrimination of roots of the two species. Through root competition barley was able to outcompete the vetch for N uptake from deeper layers. In contrast to Ofori and Stern (1987a), the increase of the amount of N resulted in a lower proportion of the barley roots compared to the vetch. Compared to a barley/vetch roots ratio of 25 at N0, the other two treatments showed lower values, both below 10.

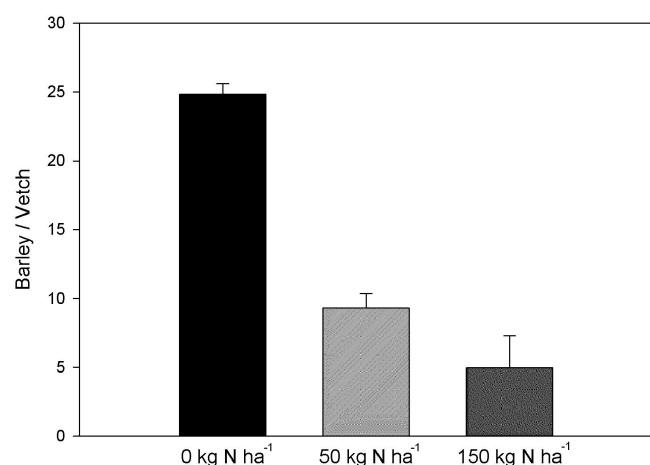


Figure 4: ratio between the number of barley and vetch roots found in samples extracted from the deep soil layers (80–120 cm) of the intercropping treatment at low (0 kg N ha⁻¹), medium (50 kg N ha⁻¹) and high (150 kg N ha⁻¹) N supply. Bars represent standard errors.

Table 1: RLD (m m⁻³) per plant at the end of the experiment in the soil layer between 0.8 and 1.2 m at different levels of N addition in the soil (average value in meter and standard errors between parentheses)

	0 kg N ha ⁻¹	50 kg N ha ⁻¹	150 kg N ha ⁻¹
Barley monocrop	321 (44) ab	318 (49) ab	220 (70) b
Barley intercrop	612 (169) a	487 (110) a	399 (75) a
Vetch intercrop	25 (6) b	52 (10) b	105 (47) c
Vetch monocrop	91 (43) b	105 (9) b	98 (54) c

Significant differences within each N level are indicated by different lower case-letters ($P < 0.05$).

Table 2: biomass at the end of the experiment in the treatments studied (g plant⁻¹) at different levels of N addition in the soil (standard errors between parentheses)

	0 kg N ha ⁻¹	50 kg N ha ⁻¹	150 kg N ha ⁻¹
Barley monocrop	3.0 (0.5) ab	3.3 (0.5) a	3.2 (0.3) a
Barley intercrop	4.2 (0.4) a	4.5 (0.7) a	4.6 (0.8) a
Vetch intercrop	0.9 (0.1) b	1.0 (0.1) b	0.9 (0.6) b
Vetch monocrop	2.6 (0.6) a	2.4 (0.6) a	2.5 (0.3) a

Significant differences within each N level are indicated by different lower case-letters ($P < 0.05$).

Table 3: N concentration at the end of the experiment (mg kg⁻¹) in the treatments studied at different levels of N addition in the soil (standard errors between parentheses)

	0 kg N ha ⁻¹	50 kg N ha ⁻¹	150 kg N ha ⁻¹
Barley monocrop	34.2 (3.5)	34.2 (2.9)	36.8 (0.6)
Barley intercrop	38.1 (3.5)	36.6 (1.7)	42.1 (1.1)
Vetch intercrop	34.4 (1.1)	35.1 (1.2)	37.5 (4.6)
Vetch monocrop	36.8 (0.6)	37.3 (1.7)	40.1 (1.7)

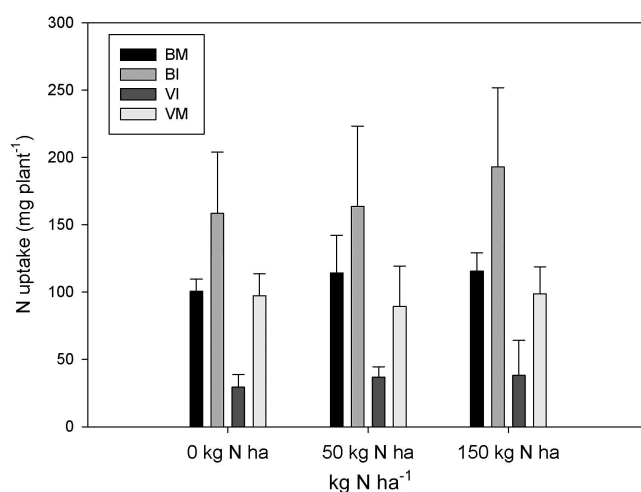


Figure 5: N uptake (mg plant⁻¹) of the harvested plants at low (0 kg N ha⁻¹), medium (50 kg N ha⁻¹) and high (150 kg N ha⁻¹) N supply. BI, barley intercropped; BM, barley monocropped; VI, vetch intercropped; VM, vetch monocropped. Bars represent standard errors.

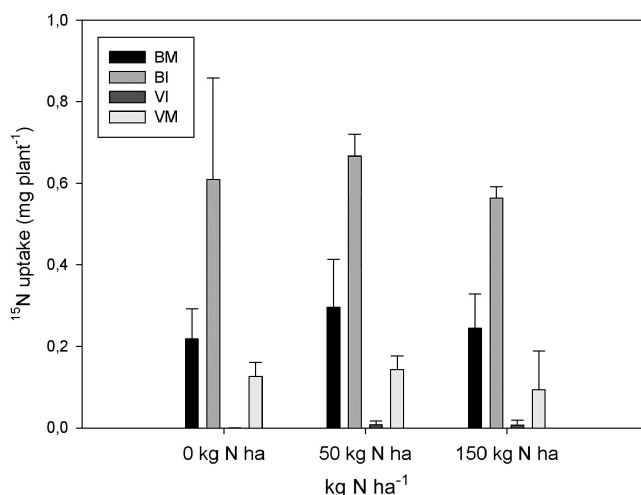


Figure 6: ¹⁵N uptake (mg plant⁻¹) at the end of the experiment at low (0 kg N ha⁻¹), medium (50 kg N ha⁻¹) and high (150 kg N ha⁻¹) N supply. BI, barley intercropped; BM, barley monocropped; VI, vetch intercropped; VM, vetch monocropped. Bars represent standard errors.

Table 4: ¹⁵N uptake per meter root (mg ¹⁵N m⁻¹ root) of the plants at the end of the experiment in the soil layer between 0.8 and 1.2 m at different levels of N addition in the soil (standard errors between parentheses)

	0 kg N ha ⁻¹	50 kg N ha ⁻¹	150 kg N ha ⁻¹
Barley monocrop	0.20 (0.02) a	0.27 (0.03) ab	0.38 (0.09) a
Barley intercrop	0.30 (0.03) a	0.44 (0.08) a	0.45 (0.10) a
Vetch intercrop	0.01 (0.01) b	0.05 (0.03) b	0.03 (0.02) b
Vetch monocrop	0.19 (0.03) a	0.40 (0.05) a	0.27 (0.07) ab

Significant differences within each N level are indicated by different lower case-letters ($P < 0.05$).

In monocrop, barley and vetch produced almost the same biomass but in the intercrop the barley was strongly dominating, producing ca. 83% of the total biomass. Other authors reported similar superior dry matter values of the non-legume compared to the legume in mixtures (Hauggaard-Nielsen *et al.* 2007; Ofori and Stern 1987b).

There was no clear influence of the N level in the topsoil on the results, and the barley N concentration was high even at N0, so the N was not a limiting factor for biomass accumulation. The high initial soil mineral N concentration explains the absence of sensitivity of the biomass to the increase in topsoil N availability. However, this might also be due to a harvest performed at an early developmental stage. Along a longer growth period the N demand would have been higher and differences in N supply could have had a much clearer effect. These results confirmed the lack of influence of the N level in the biomass or N accumulation in legume/non-legume intercrops. Other authors like Andersen *et al.* (2004) and Jensen (1996b) reported similar findings.

Unlike the biomass production, the RI of barley in monoculture was two to three times higher than the root density of vetch, whereas in the intercropping, root density was close to the monocrop barley. Also the RD of the barley and barley-vetch intercrops was around 50% deeper than that of vetch as commonly reported by other researchers (Corre-Hellou *et al.* 2007; Thorup-Kristensen 2001). This shows a deep root foraging strategy in the barley and intercropping treatments, while the sole-cropped vetch was not able to exploit these layers. The observed differences in root dynamics between the two species may be a result of their different foraging strategies and dependence on soil available N. The barley tended to reduce its RI in the monocropping treatment with increased N, while the intercropping treatment showed more stable values at the different N levels, probably due to a compensation produced by the increase of the vetch roots number. In the deepest soil layers, the intercropping produced slightly more roots than the monocropped barley, i.e. the deep RLD per barley plant was more than doubled in intercropping. The intercropping treatment enhanced the roots production of the barley at deep soil layers, while the vetch roots tended to be less present. A reason for this behavior could be that the barley is more competitive than the vetch in its foraging strategy, and thus its roots dominated in the intercropping. In the monocropping treatment, the barley roots growth was limited in a more balanced competition with a plant of the same species. The competition for soil N since early stages could have influenced the root growth in the mixture. This is an important issue for the initial growth, the establishment and also the further development of the plants in the cereal and legume intercrops (Trannin *et al.* 2000). Even without statistical significance, while the barley and intercropping treatments tended to reduce the RLD with the increase of topsoil N, the sole-cropped vetch attained slightly higher values at higher N rates. Analyzing the roots extracted from the 0.8 to 1.2 m layer microscopically showed that barley roots

dominated in this soil layer and more so than would be predicted based on the monocrops. Surprisingly, the fraction of vetch roots in this soil layer was increased at high N supply. Even without statistical significance, the RLD results in the monocrops are in agreement with the root discrimination. Other authors found an increasing dominance of the non-legume with higher N levels (Corre-Hellou *et al.* 2007; Jensen 1996b).

Our results could be due to a reduction in the competitive response of the barley in an N-abundant growing medium (Andersen *et al.* 2004). Another explanation is an increase in the competitiveness of the vetch when a large pool of N is available in the soil, which may be a less resource-consuming strategy compared to N fixation (Izaurrealde *et al.* 1992). The ratio between barley and vetch roots increases from 4% at N0 to 20% at N2. This is in line with the RLD of the two monocrops in the soil layer between 0.8 and 1.2 m because while the vetch roots at N0 is 28% of the length density of the barley roots, at N2 it increases until 44%.

Easy and reliable methods for plant root discrimination do not exist for non-woody plant species, although a range of methods such as labeling approaches, molecular markers, anatomical keys and infrared absorbance spectra and other chemotaxonomic methods have been developed (review by Rewald *et al.* 2012). Recently, roots from a cereal crop could be discriminated from dicot weed by their chemical composition using Fourier transform infrared spectroscopy–Attenuated total reflection (FTIR–ATR) spectra (Naumann *et al.* 2010), however for a limited number of samples. Our study shows how root microscopy is a useful, although laborious, technique for clear discrimination between species in samples from intercropping and relevant to a broad range of legume/non-legume mixtures.

The results showed little N limitation to crop growth, as the increase in growth and N uptake with increasing N supply was small, only 20–50% of the added N was taken up, by the barley that showed even at N0 a high N concentration. The barley attained values around 3.5 and 4% of N, respectively, for monocrop and intercrop treatments, while the vetch 3.8 and 3.5%, for the same situations. These values are in accordance with the previous results also from field studies (Tosti *et al.* 2012 for the intercropping situations; Ranells and Waggener 1996 for the sole-cropped) and reveal a sufficient nutrition of the plants even at the low N level. Intercropped barley showed the highest N uptake per plant at all N levels compared to the sole-crop situations tested. The vetch as partner in the mixture was more limited in the N uptake than in biomass, while barley responded in the opposite way. This result can be caused either by direct resource competition, if barley is taking the nitrogen from the soil before vetch is able to do so (Trannin *et al.* 2000), or by barley reducing the root growth of vetch through root avoidance or other effects (Andersen *et al.* 2014), and thereby its ability to take up the available N from the soil. Semchenko *et al.* (2007) pointed that root avoidance might be caused by root-mediated allelopathy and non-toxic signals that are elicited when

root contact occurs. The results on ^{15}N uptake per meter root show that the uptake activity of vetch was strongly reduced in the intercropping compared to monocrop. The acquisition of resources by a competitor's roots creates depletion zones in the soil and thus generates a more heterogeneous pattern of resource distribution than when plants are grown alone. We do not have an explanation for this very clear reduction in N uptake activity by the vetch roots in intercropping, but in some way the presence of the barley roots strongly reduced the uptake activity of the vetch roots in this layer. This might be related to an enhanced ability of the cereal component to utilize soil N pools (Høgh-Jensen and Schjoerring 1997). The much higher per plant RLD of barley in intercrops compared to monocrops may explain the ^{15}N uptake results, as the intercropped barley reached up to three times (0.6 mg plant^{-1}) the values of the monocropped barley. In monocrops, the higher uptake by barley appears to be mainly the effect of the differences in root length in deep layers. These results confirmed the hypothesis that the intercropping produced deeper and more intense roots than the sole-cropped barley and vetch. The mixture also increased the N uptake from deep soil layers. However, as the initial amount of N in the soil was already high, the root growth and the N uptake were not significantly affected by the N increase.

Facilitation takes place when plants ameliorate the environment of their neighbors and increase their growth and survival (Hauggaard-Nielsen and Jensen 2005). The difference between monocrops and intercrops slightly decreased with the increment in topsoil N, as the plants may use the topsoil N instead of extracting it from deeper layers. Roots would not exploit subsoil resources as much as they could do according to their root growth there, if there is a high amount of available N in the topsoil (Rowe *et al.* 2001). This is in accordance also with the root discrimination results from the intercrop, which showed this tendency for barley, but the contrary for the vetch.

CONCLUSIONS

The barley was dominant in the intercropping, as typically found. Root growth in the intercrops was similar to root growth in the barley monocrops and much stronger than in the vetch monocrops. In the deepest soil layers, the intercropping tended to produce more roots than the monocropped barley, and expressed per plant, the intercropped barley plants produced twice the root length of the monocropped barley plants. The N level did not affect the roots growth much but influenced the roots ratio between species at deep soil layers in the intercropping. The ratio decreased at higher N levels, and though the barley root system was still dominant in the mixture, the presence of vetch roots increased with N. This surprisingly result was confirmed with the RLD results in deep layers of the monocrops. The discrimination method performed in this study relies on the anatomical and morphological differences observed between dicots and monocots and proved to be a reliable method providing valuable data for the analysis.

The intercropping influenced also the N uptake of the plants, inducing an increase in the uptake of the barley and decreasing the one of vetch, compared to monocrops, as confirmed by the ^{15}N results. In accordance with the roots results in the deep layers, the barley took up more N than the vetch, outcompeting the latter through root competition. Most differences in ^{15}N uptake from the deeper soil layers (monocropped vs. intercropped barley, and monocropped barley vs. monocropped vetch) seem to be related to differences in RLD in the deeper soil layers, but the ^{15}N uptake of vetch in intercropping was reduced much below the level expected due to its reduced RLD in the deep layers.

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